A STUDY OF THE TOXICITY AND STABILITY OF DRIED MOCCASIN (Agkistrodon piscivorus) VENOM

C. B. POLLARD, A. F. NOVAK, R. W. HARMON, AND W. H. RUNZLER University of Florida

Since the availability of poisonous snakes in some localities is somewhat seasonal, it is desirable to collect, process, and store the venom for future use.

Although the practice of drying venom has been employed for many years, the literature affords little information concerning the toxicity or the relative stability of the dried venoms. In view of this situation, a study of the toxicity and the stability of dried moccasin (Agkistrodon piscivorus) venoms was undertaken.

A limited number of experiments here have shown that the toxicity of the venom from individual snakes of the same species varies even though the snakes are approximately the same size.

In order to secure relatively uniform samples of venom for experimental use, the venom from fourteen to eighteen moccasins of uniform size was pooled. Eleven of these pooled samples were prepared. Each pooled sample was thoroughly mixed and then centrifuged to remove sediment. The clear, supernatant liquid was separated from the sediment and was then dried at room temperature at 4-8 mm. Hg. All fresh venom was kept at $15^{\circ}-20^{\circ}$ C. while awaiting processing, and all samples were dried within seventy-two hours after "milking."

Doses of 0.5 gm./kg. of the sediment, injected intraperitoneally, failed to produce death of any test animal, although severe diarrhea was observed in animals receiving this substance. This condition may have been caused by magnesia present in snake venom. The sediment was discarded since it was not toxic.

Method of Assay

There have been a considerable number of biological assay techniques developed which could be employed for the determination of the toxicity of venom. In these studies, it was necessary to have a method which would enable detection of a small loss in potency of the toxic principles of the venom. Several variations of previously investigated methods were studied. The white rat was selected as the test animal since best results were obtained with this species. By determining the MLD and LD_{50} values of the original dried, pooled samples, it was a relatively easy task to determine the LD_{50} for each sample after it had been stored at 15°-20° C. for periods of 6, 12, 18 and 24 months.

White rats from eight to twelve weeks of age, showing no pathological defects, were placed on a standard feed which contained all known growth factors. No nutritional deficiencies or clinical manifestations of disease were observed on any rat which was selected for inoculation with venom samples.

Various concentrations of venom were prepared by dilution of the dried venom with physiological saline solution (0.89% sodium chloride). The rats were placed upon an inoculation board and an intraperitoneal injection of the diluted venom samples was administered, using a sterile one-inch hypodermic needle. The rats were then individually caged and observed. Feed and water were supplied *ad libitum*. Six to twelve rats were used for each determination.

The results of these studies are shown in TABLE 1.

		•	,		
Sample No.	LD50 Initial	LD ₅₀ After storage for 6 months	LD ₅₀ After storage for 12 months	LD ₅₀ After storage for 18 months	LD ₅₀ After storage for 24 months
$ \begin{array}{c} 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7 \\ 8 \\ 9 \\ 10 \\ 11 \\ 1 \end{array} $	$29 \\ 28 \\ 36 \\ 41 \\ 45 \\ 42 \\ 39 \\ 38 \\ 34 \\ 29 \\ 45 \\ 45 \\ 100 $	$\begin{array}{c} 31 \\ 32 \\ 34 \\ 38 \\ 44 \\ 45 \\ 37 \\ 43 \\ 38 \\ 34 \\ 42 \\ 42 \\ \end{array}$	$\begin{array}{c} 85\\ 33\\ 42\\ 41\\ 44\\ 53\\ 41\\ 50\\ 36\\ 35\\ 49\end{array}$	$\begin{array}{c} 35\\ 31\\ 37\\ 39\\ 45\\ 44\\ 39\\ 42\\ 40\\ 32\\ 52\end{array}$	$\begin{array}{c} 34\\ 30\\ 38\\ 40\\ 44\\ 46\\ 41\\ 41\\ 36\\ 32\\ 50\\ \end{array}$

TABLE 1

Data Concerning the Toxicity and Stability of Moccasin (Agkistrodon piscivorus) Venom

LD₅₀ in mg./kg.

SUMMARY

It is apparent that the toxic portion present in the pooled and dried samples of moccasin venom tested in these investigations is relatively stable over a period of twenty-four months when stored at $15^{\circ}-20^{\circ}$ C. The slight difference in the LD_{50} values might be attributed to variations in animals and experimental errors.

ACKNOWLEDGMENT

A portion of this work was supported by the United States Public Health Service.

Venom used in this investigation was obtained from the Ross Allen Reptile Institute, Silver Springs, Florida.

Quart. Journ. Fla. Acad. Sci., 15(3), 1952.